CHAPTER-II

JH MIMICS FROM THE WOOD OF *Cedrus deodara* LOUD.

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ABSTRACT

This Chapter describes the investigation carried out on the acetone extract of wood of *Cedrus deodara* in order to locate the JH active components. The considerable JH activity observed in the acetone extract was located on two major components of the acidic fraction *viz*. 7(8)-dehydrojuvabión (X) and 7-hydroxyjuvabion (XIII). In all, seven acids were isolated as methyl esters and characterised essentially on the basis of spectroscopic evidence.

JH MIMICS FROM THE WOOD OF CEDRUS DEODARA LOUD.

INTRODUCTION

The genus Cedrus (family; Pinaceae; sub-family; Laricoideae) consists of four species[1]: (1) Cedrus deodara Loud (2) Cedrus libanotica Link (3) Cedrus atlantica Mannet and (4) Cedrus brevifolia Hook. F.

Cedrus deodara[2,3] ("Deodara" in Sanskrit; "Deodar" in Hindi, Marathi and Kashmiri), the Himalayan Cedar is a native of the Himalayan mountain region. The tree grows in the northwestern Himalayas, Kurum valley and Chitral, at an elevation of about 1400-3500 m[3]. It is a beautiful and graceful tree of pyramidal outline growing to a height of about 50 m. Its leading shoots and extremities of branchlets are drooping; the foliage is dark green or bluish green;[4] the leaves are three-sided, solitary, on elongated shoots, but in dense clusters on arrested branchlets, catkins are cylinderic and single; the cone is erect, 10-13 cm long; scales are thin, rounded and the seeds are winged.[2] The wood is aromatic, oily, not attacked by white ants, moderately hard and durable. It is one of the most highly valued timbers of India.[2] The wood on steam

distillation yields an essential oil with a pleasant balsamic odour.

The essential oil of C. deodara has been the subject of investigation by a number of authors. A review of their results is presented later in the Thesis (Chapter V).

JH activity of wood extractives

It had been reported that acetone extract from the wood of C. deodara shows considerable Juvenile hormone (JH) activity towards Dysdercus koenigii. Screening of the total essential oil from C. deodara as well as several individual compounds (α and β-himachalene, himachalol, atlantone etc.) showed[5] that though a few of these do mimic JH activity, the activity is much less than that of the total wood extract.

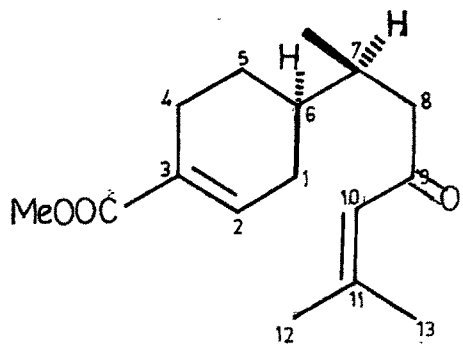
Subsequently, it was found[6] that the wood of C. deodara contained a number of sesquiterpene acids and the JH activity resided in these compounds. Analysis[6] of the acidic portion as methyl esters yielded (+)-10,11-dehydrojuvabion (1) and two mixtures, one reportedly

containing (II) and (III) and another containing (IV) and (V) and (VI). Though all possible methods (column chromatography of acids, separation of acids *via* cyclohexylamine salts and various derivatives, preparative GLC of esters etc.) were employed to isolate the individual components; the desired separations could not be achieved. However, from the previous work it could be concluded that to achieve the objective of isolating pure compounds the following points have to be kept in mind:

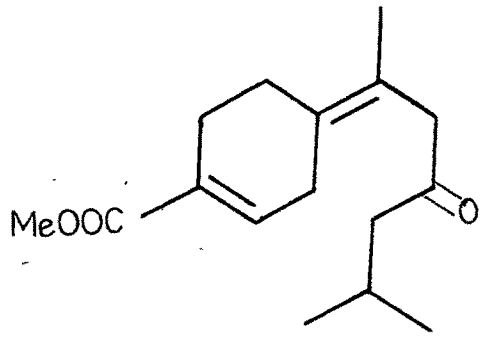
1) It is easier to handle the methyl esters than the acids and that any investigation should use the total methyl esters as starting mixture.

2) The isolation procedure should be so planned as to avoid all conditions that might lead to formation of artefacts.

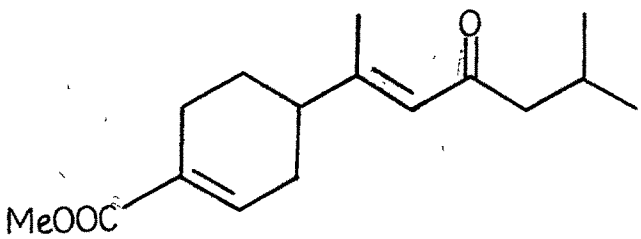
These factors were taken into consideration in the present investigations.



(I)



(II)



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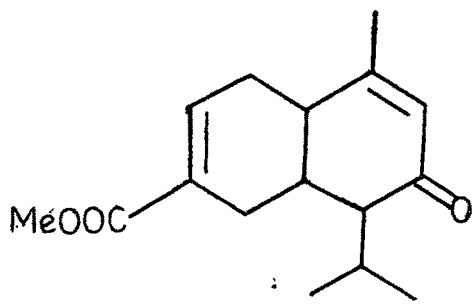

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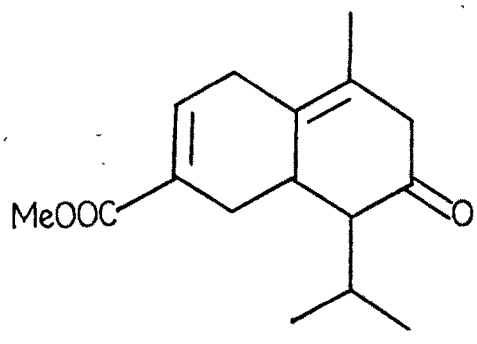

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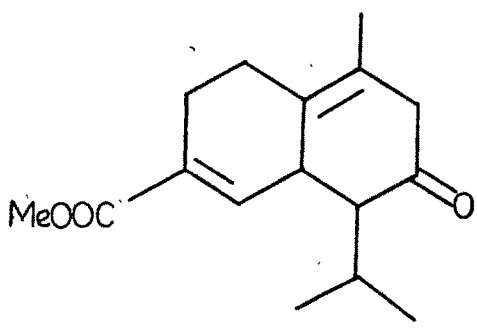


(VI)

FIG · 1

PRESENT INVESTIGATION

Cold percolation of the powdered wood of C. deodara with acetone gave an extract (1.8%) which after solvent stripping was mixed thoroughly with celite. Cold percolation of this celite-extract mixture with petroleum ether gave petroleum ether solubles (73%), the JH activity of which was determined using 4 hr old instar nymphs of the red cotton bug, Dysdercus koenigii, as test insects. Farnesyl methyl ether was used as the standard. The results are summerised in Table 1. The pet. ether extract was segregated into the neutrals and acidic parts in the usual manner.

Table 1 : JH activity of wood extractives

Compound	Dose in ug		
	10.0	1.0	0.1
	Score*		
Acetone extract	2.2	0.0	-
Total pet. ether extract	1.5	0.0	-
Neutrals	0.5	0.0	-
Crude esters	3.4	1.5	-
Farnesyl methyl ether	1.5	0.0	0.0

*Score represents mean of two experiments, each of which was carried out using three replicates of 15 nymphs each. Nymphs which successfully moulted were characterised and scored as: normal adults (0); adult nymphs (1); intermediates (2); nymph-adults (3) and sixth instar nymphs (4).

In view of the enhanced JH activity of esters they were analysed for individual components.

SEPARATION OF ACIDS

The total acid part was converted into the corresponding methyl esters (CH_2N_2 method). Fig. 2 shows the HPLC elution profile of the total esters using UV 254 nm detector. (All the esters isolated during prior work absorb in 254 nm region due to α,β-unsaturated ester chromophore). It becomes clear on the basis of HPLC (Fig. 2), TLC (Fig. 3) and GLC (Fig. 4) that the total esters are composed of one major and many minor components.

The esters were chromatographed over a column of silica gel to give two esters in pure state. Spectroscopic evidence (see expt.) identified the two as methyl esters of oleic (VII) and tetracosanoic acid (VIII).

$$CH_3 - (CH_2)_7 - CH = CH - (CH_2)_7 - COOCH_3$$

(VII)

$$CH_3 - (CH_2)_{22} - COOCH_3$$

(VIII)

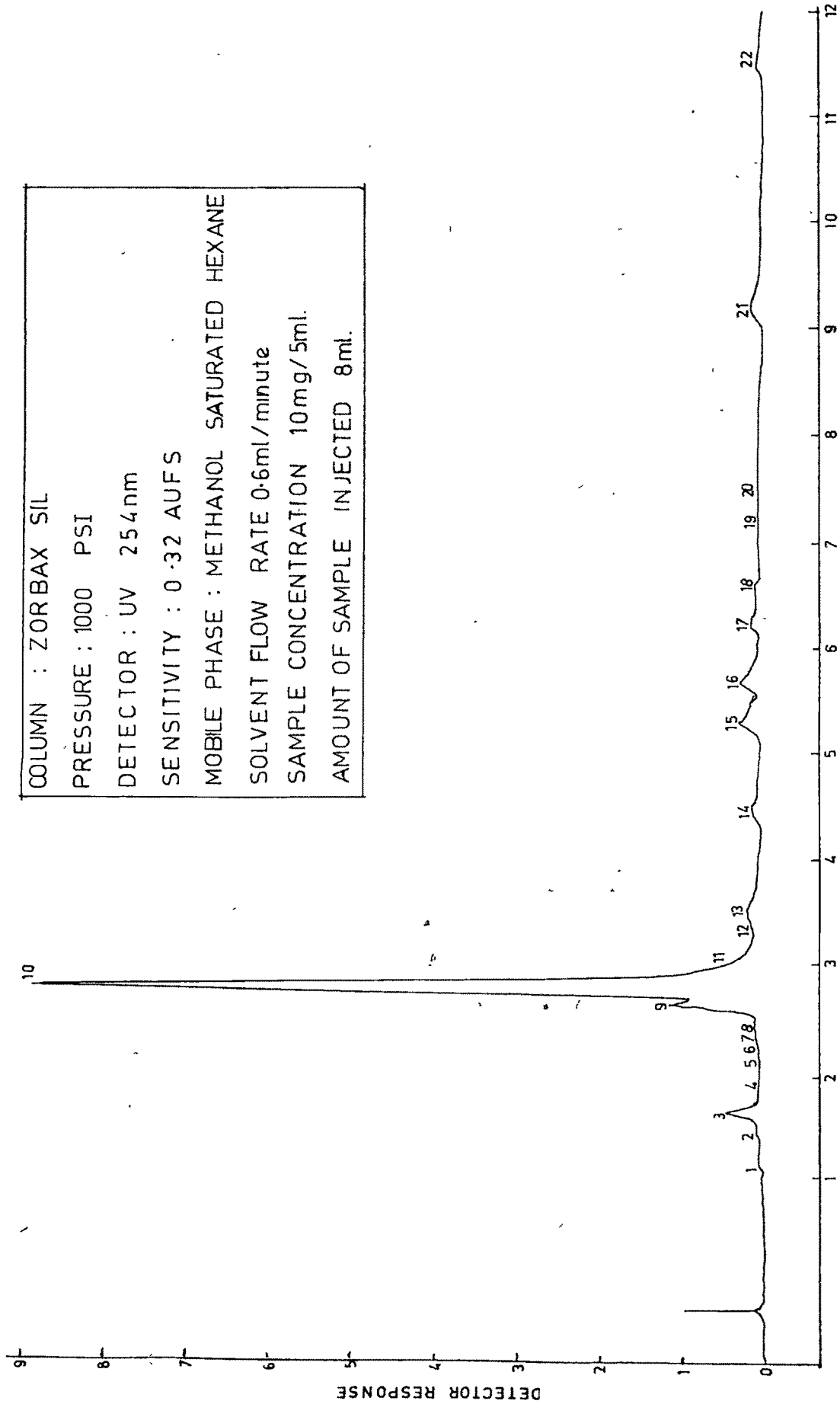


FIG-2: HPLC OF TOTAL ESTERS

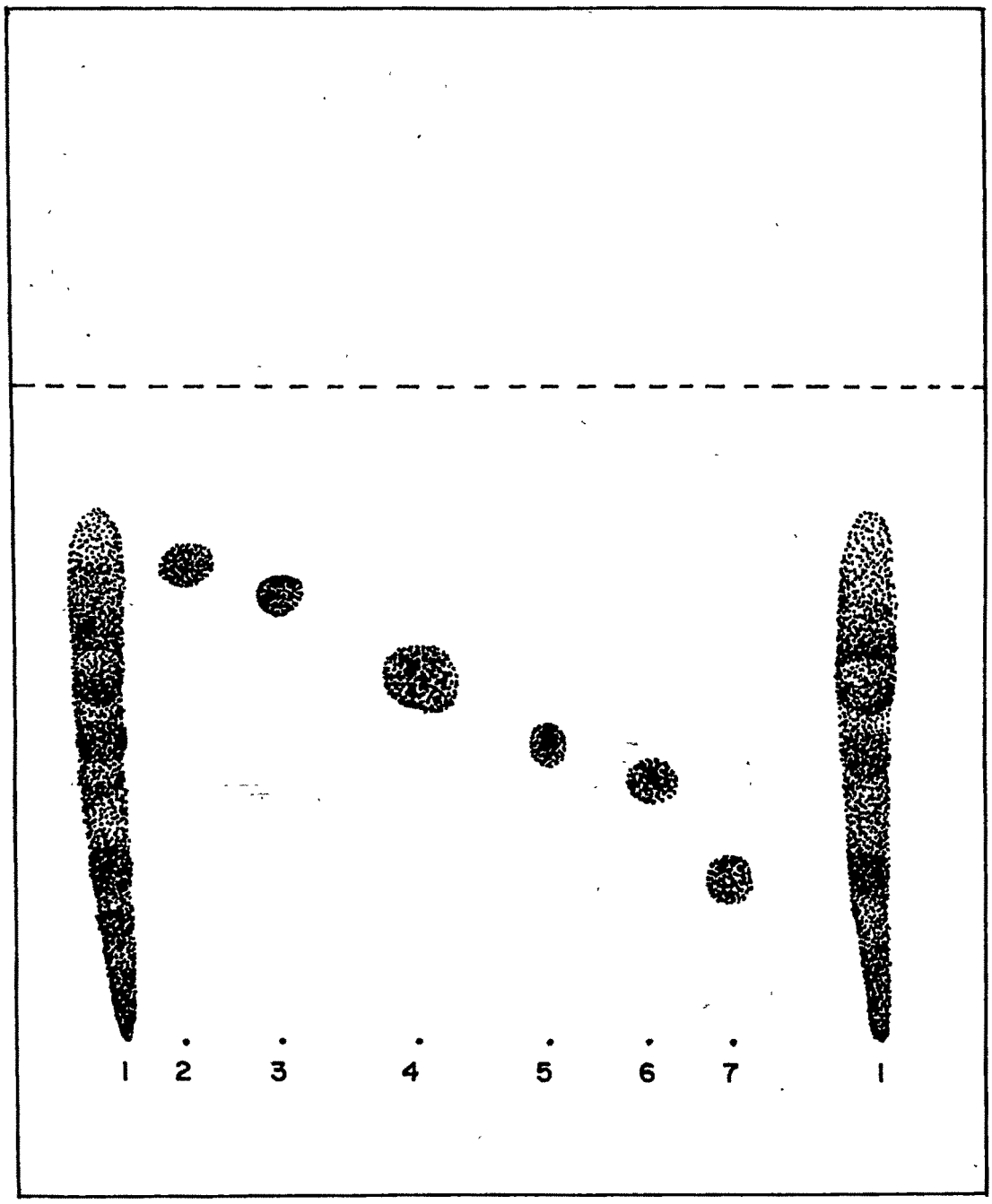


FIG. 3 : TLC OF COMPONENTS FROM TOTAL ESTERS
SILICAGEL PLATES SOLVENT SYSTEM :
(50 % ETHER / LIGHT PETROL)

(1) TOTAL ESTERS (2) METHYL OLEATE
(3) METHYL 4 – (R) – LIMONENE 8(E) – 9 – CARBOXYLATE
(4) 7(8) – DEHYDROJUVABION (5) METHYL GERONATE
(6) METHYL 4 – ACETYL – 1 – CYCLOHEXENE CARBOXYLATE
(7) 7 – HYDROXY JUVABION

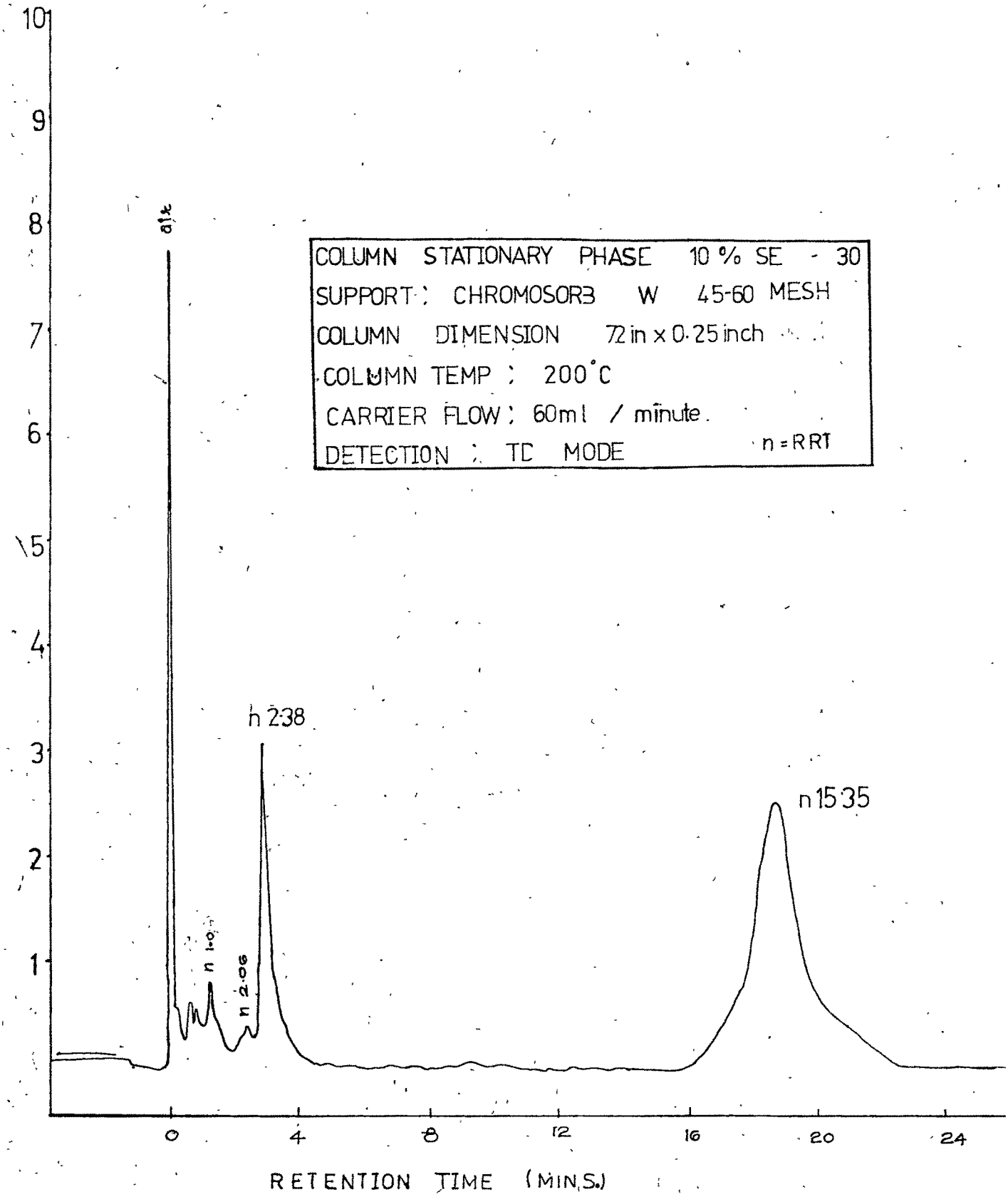


FIG-4 : GLC OF TOTAL ESTERS

Further chromatography failed to give any pure compound. Subsequently, the total esters were distilled and both the residue and distillate tested for JH activity (Table II) as described earlier (page 30).

Table II : JH activity of esters towards *D. koenigii*

Name	Dose in ug		
	10.0	1.0	0.1
	Score*		
Total esters	3.4	1.5	0.0
Distilled esters	3.3	1.4	0.0
Residue	3.0	1.0	0.0
Farnesyl methyl ether	2.0	0.0	0.0

*For details of scoring see Table I.

Distilled Esters

The distilled esters were chromatographed on Medium

Pressure Liquid Chromatograph (MPLC) using as eluent chloroform with increasing percentage of ethyl acetate to effect a broad separation into seven fractions (total recovery 97%). Fractions 1,2,3 (total 13% of eluate) consisted mainly of fatty acids and were not investigated further (Chart I). Fr. 4 on chromatography over silica gel gave following compounds:

(+)-Methyl 4(R)-limonen-8(E)-9-carboxylate (IX): (TLC: R_f, 0.65; GLC: RRT, 1.0 ; HPLC: peak No. 3). The fraction eluting with 4% diethyl ether in pet. ether was found to be homogenous. The distilled compound, a colourless liquid $C_{12}H_{18}O_2$ (M^+: m/e 194) shows following spectral features:

IR (Fig: II-1): 1720, 1640, 1230, 1210, 800 cm^{-1}.
PMR (Fig: II-2): 5.61 ppm (1H, s, -C=C<u>H</u>-COOMe); 5.35 ppm (1H, unresolved triplet, -C=C<u>H</u>-CH_2-); 3.61 ppm (3H, s, --COO<u>CH_3</u>); 2.12 ppm (3H, s, <u>CH_3</u>-C=CHCO_2Me); 1.63 ppm (3H, s, <u>CH_3</u>-C=CH). These spectral features are in accordance with the structural requirements of methyl esterof(+)-limonene carboxylic acid (IX) isolated from <u>C. deodara</u> earlier.[7]

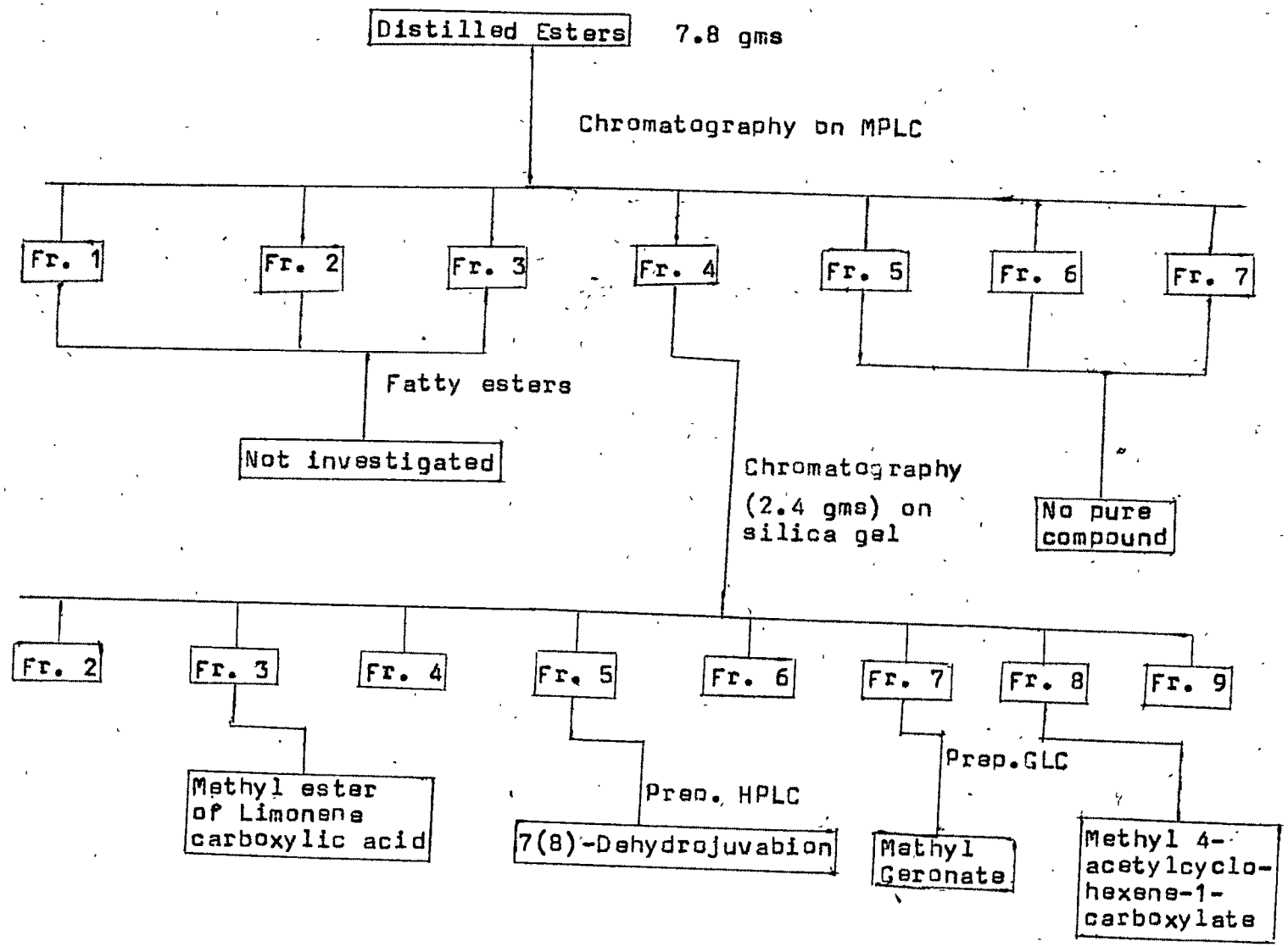
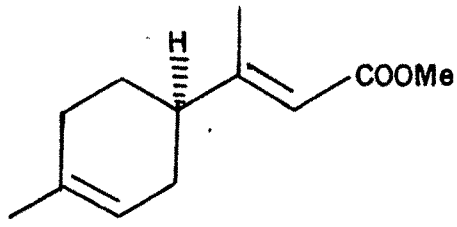

Distilled Esters
7.8 gms
Chromatography on MPLC
Fr. 1
Fr. 2
Fr. 3
Fr. 4
Fr. 5
Fr. 6
Fr. 7
Fatty esters
Not investigated
Chromatography
(2.4 gms) on
silica gel
No pure
compound
Fr. 2
Fr. 3
Fr. 4
Fr. 5
Fr. 6
Fr. 7
Fr. 8
Fr. 9
Methyl ester
of Limonene
carboxylic acid
Prep. HPLC
7(8)-Dehydrojuvabion
Prep.GLC
Methyl
Geronate
Methyl 4-
acetylcyclo-
hexene-1-
carboxylate

CHART I



(IX)

Comparison of the electron induced fragmentation pattern of the ester with that reported of the acid earlier[7] confirmed the structure.

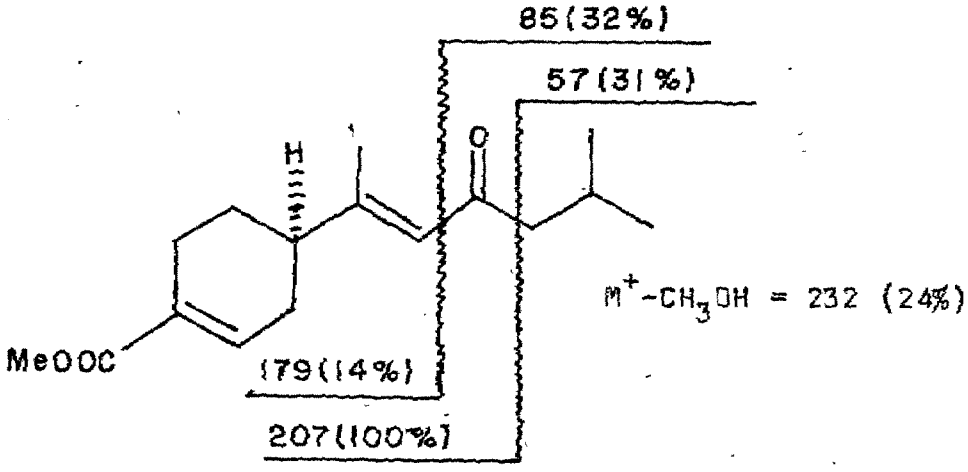
(+)-7(8)-Dehydrojuvabion (X): (TLC: R_f, 0.55; GLC RRT, 15.35; HPLC Peak No. 10).

The major fraction eluted with 4% ether/pet. ether showed only a single peak on GLC (10% SE-30, 200°C). However, by HPLC column (Zorbax sil) it was found to be impure. This compound was purified by preparative HPLC on a spherosil column using MeOH-hexane as eluent to give essentially TLC, GLC and HPLC pure compound. This compound analyses for $C_{16}H_{24}O_3$ (M^+, m/e 264). The IR spectrum (Fig: II-3) of this compound shows that it is an unsaturated keto-ester (C=O, 1712 and 1685 cm^{-1}, C=C, 1648 cm^{-1}). This is supported by its UV spectrum (λ_{max}^{EtOH} : 237 nm, ε= 11040).

Its PMR spectrum (Fig: II-4) shows a 6H doublet centered

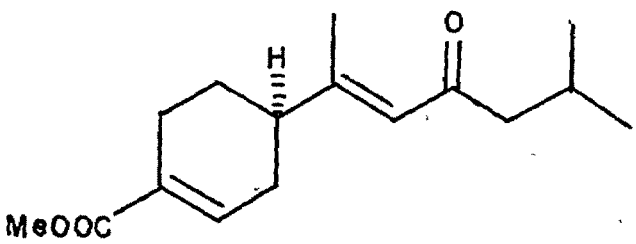
at 0.91 ppm (J = 7 Hz) assignable to $\underline{CH_3}$-CH-$\underline{CH_3}$; a 3H singlet at 3.67 ppm (-COO$\underline{CH_3}$) and two singlets at 5.97 and 6.91 ppm (1H each) assignable to olefinic protons in α- and β-positions respectively of α,β-unsaturated carbonyl compounds and a sharp singlet at 2.10 ppm assignable to a methyl group as in $\underline{CH_3}$-C=C-. The above spectral data indicates that the compound under discussion may be 7(8)-dehydrojuvabion (X).

This conclusion finds support in the electron induced fragmentation of this compound as depicted below. This is

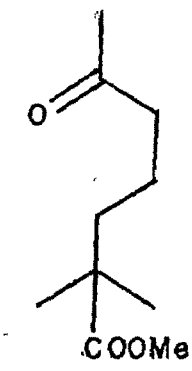


the first reported instance of the isolation of this compound in pure form from nature; the compound however, had been synthesised earlier.[8]

Burger et al.[8] had synthesised (-)-7(8)-dehydrojuvabion starting with (-)-methyl perillate having S-configuration at the asymmetric centre. Since the compound isolated from C. deodara has a positive sp. rotation (+ 23.4° C. 2.5%), the absolute stereostructure of this compound should be as depicted in(X).



(X)



(XII)

Methyl geronate (XI): (TLC: R_f, 0.45; GLC: RRT, 2.06).

The fraction eluting with 8% ether/pet. ether was impure and attempted purification by repeated chromatography failed. Eventually, purification by preparative GLC gave a TLC pure compound.

This compound ($C_{10}H_{18}O_3$, M^+, m/e 186) displays in its IR spectrum (Fig: II-5) absorptions at 2945, 1722, 1135, 1165, 1195 and 1360 cm^{-1}. Its PMR spectrum (Fig: II-6)

shows following features: $(C\underline{H}_3)_2$-C-COOCH$_3$ (6H, s, 1.15 ppm); $C\underline{H}_3$-C=O (3H, s, 2.045 ppm); -COO$\underline{Me}$ (3H, s, 3.625 ppm); -$C\underline{H}_2$-CO (2H, m, 2.28 ppm); 1.32-1.5 (4H, m).

On the basis of above structural features, the compound was identified as methyl geronate (XI). The structure was confirmed by mass spectral fragmentation pattern identical with the reported pattern.[9]

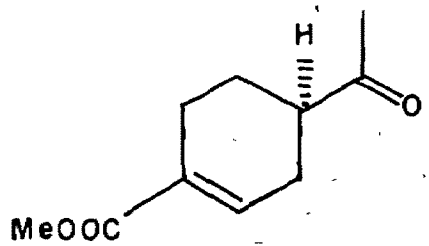
Methyl geronate has been obtained as a degradation product of carotenes[10] and also during ozonolysis of β-himachalene.[11] The genesis of the compound in C. deodara is uncertain.

Methyl 4(R)-acetyl-1-cyclohexene-1-carboxylate (XII): R_f, 0.39. GLC: RRT, 2.38; HPLC peak No. 15

The fraction eluting with 10% ether/pet. ether was homogenous on TLC (50% ether/pet. ether). This compound ($C_{10}H_{14}O_3$, M^+ m/e 182) is clearly an α,β-unsaturated ester as is evident from its UV (λ_{max}^{EtOH} 220, ε = 6100). Its PMR spectrum (Fig: II-7) displays a signal in olefinic region at 6.93 ppm (1H, s) characteristic for the β-proton of α,β-unsaturated ester group viz. C$\underline{H}$=C-COOCH$_3$; present

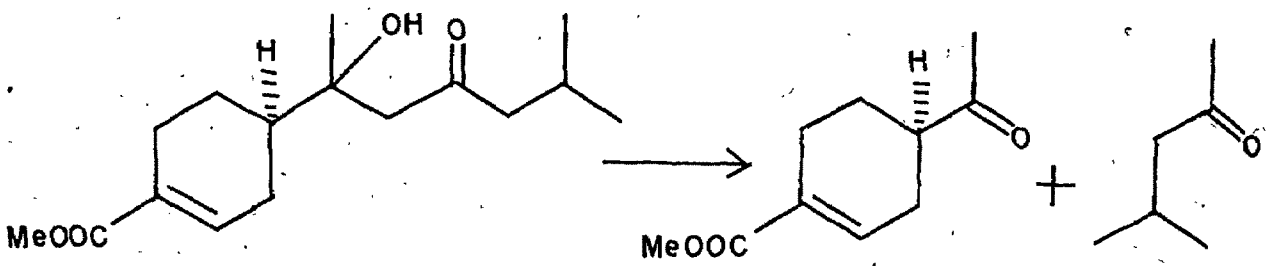
in all the C. deodara acids identified so far and a sharp singlet at 3.69 ppm (3H) for the $-COOCH_3$ protons. The compound also displays a sharp 3H singlet at 2.14 ppm attributable to CH_3-CO.

In its IR spectrum (Fig: II-8) the compound displays absorotions at 2960, 1715, 1700, 1260, 1170 and 1040 cm^{-1}. The above spectral features point out that the compound under discussion may be (XII). This normonoterpenic ester



(XII)

apparently is an artefact arising from the retro-aldol fragmentation of the tertiary alcohol (XIII) which was later isolated from the acidic fraction. The stereochemistry

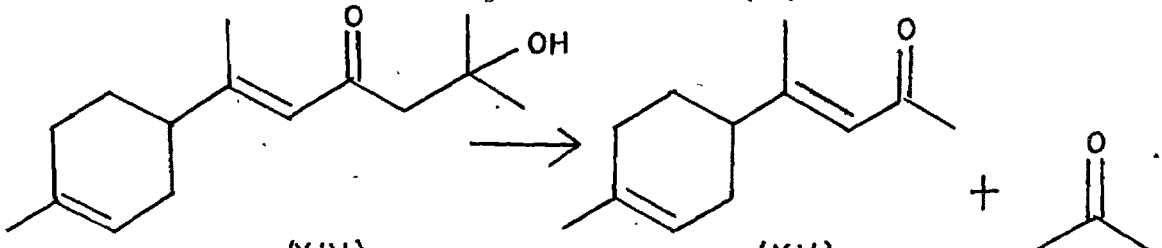


(XIII) (XII)

of XII and C-4 thus follows as that in(XIII).

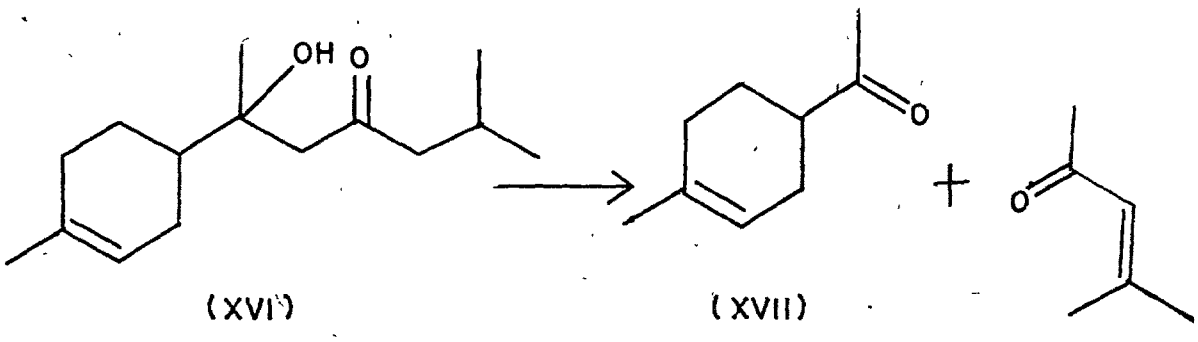
Analogous fragmentations are:

i) Fragmentation[13] of atlantolone (XIV) on GLC column to give the enone(XV).



(XIV) (XV)

ii) Fragmentation[13] of the tertiary alcohol(XVI) to give 4-methyl-Δ^3-tetrahydroacetophenone (XVII).



(XVI) (XVII)

<u>7-Hydroxyjuvabion (XIII)</u>:(TLC: R_f, 0.23)

Isolation of the normonoterpenic ester (XII) led

us to expect that the tertiary alcohol (XIII) could be one of the constituents of the total esters. Its structure reveals it is a highly labile compound susceptible to fragmentation under basic and thermal conditions. Several attempts to isolate this precusor to both (X) and (XII) did not frutify. Finally, this goal was achieved by following the isolation procedure outlined below.

The total light petroleum ether solubles was extracted with 5% sodium carbonate to give on usual work-up at 2°C acids (8.9%) which were immediately esterified (CH_2N_2). Flash chromatographic separation[14] of the esters over a column of SiO_2 (Grade IV A) gave fractions which were monitored by IR for -OH absorbance.

The fraction eluting with 20% ether/pet. ether was found rich in -OH content and subsequently purified by preparative TLC to give a TLC pure (R_f 0.23 50% ether/pet. ether) compound.

This compound ($C_{16}H_{26}O_4$, M^+, m/e 282) reveals in its IR spectrum (Fig: II-9) absorptions for hydroxyl (3500, 1080 cm^{-1}) and ester (1710, 1250 cm^{-1}) functionalities. In its PMR spectrum (Fig: II-10) the compound displays a signal

at 6.90 ppm (1H, s) characteristic of olefinic proton belonging to α,β-unsaturated ester system *viz.* $\underline{CH}-C\div COOCH_3$ of dehydrojuvabion skelton (X).

A sharp singlet centered at 3.66 ppm (3H) is attributed to $-COO\underline{CH_3}$. The spectrum further shows absorptions at 1.13 ppm (3H) which can be assigned to protons of a methyl group attached to carbon carrying a tertiary hydroxyl function. A doublet at 0.93 ppm (6H, d, J = 7Hz) reveals the presence of two geminal methyl groups in the compound. Signals at 2.23 ppm (2H) and 2.47 ppm (2H) are attributable to $-CO-\underline{CH_2}-C-$ and $-C(OH)-\underline{CH_2}-CO-$ grouos respectively. All this spectral evidence clearly point towards the structure (XIII) assigned for the isolated alcohol.

The thermal lability of the compound also supports the above structure. Although homogenous by tlc, the compound partly decomposes to give the keto ester (XII) when analysed by GLC (5% carbowax, 150°C). Attempt to distil the compound also led to its fragmentation (NMR) to the keto ester (XII).

Chemical evidence advanced in confirmation of the stereostructure (XIII) is given below:

Treatment of the compound with slightly acidic (pH-4) silica gel (grade IIA) in ether for 2 hrs followed by filtration and solvent removal gave a dehydrated product identical in all respects (TLC, GLC, IR and NMR) with 7(8)-dehydrojuvabion, isolated from C. deodara as described earlier. This result confirms the stereochemistry at C-4 but leaves undecided that at C-8.

JH activity of isolated esters

The JH activity of the two sesquiterpenic esters isolated from C. deodara was determined using 4 hr old last instar nymphs of D. koenigii as usual. The results are given in Table III.

Table III: JH activity of isolated esters

Compound	Dose in ug		
	10.0	1.0	0.1
		Score*	
Dehydrojuvabion(X)	3.5	1.5	0.0
Tertiary alcohol(XIII)	3.5	1.5	0.0
Farnesyl methyl ether	2.0	0.0	0.0

*For details of scoring, see Table I.

It is now clear that the JH activity of C. deodara extract is essentially due to the major acid components of the extract viz. 7(8)-dehydrojuvabion (X), and 7-hydroxyjuvabion; both of which have the same degree of JH activity, which is several times higher than that of farnesyl methyl ether.

Chemical constituents of the distillation residues

Since the distillation residue showed JH activity (Table II), this was also examined. IR spectrum of the complex mixture (not distilled at 240°C/0.005 mm in Kieglrohr unit) showed absorptions due to OH (3450, 1080 cm^{-1}) C=O (1710, 1245 cm^{-1}) and $-CH_2-$ (2940, 1430 cm^{-1}) groupings. The PMR spectrum showed large comolex signals due to methylene protons (1-2.5 ppm; 55% of all the protons) signals of tertiary methyls (0.89, 0.94 ppm), $-COOCH_3$ (3.65 ppm) $-CH_2-O$ (3.4-4.0 ppm) groupings and very weak signals for olefinic protons (5.0-6.5 ppm).

TLC analysis of this fraction using several solvent systems (pet-ether/ether; chloroform-ethyl acetate; acetone, benzene-ethylacetate, benzene-methanol) showed

only long streaks; no individual spots could be identified. Chromatography over a column of silica gel did not result in any worthwhile separation. TLC analysis of chromatographic fractions also showed only streaks and no identifiable spots. This was therefore not further pursued.

EXPERIMENTAL

All melting points and boiling points are uncorrected. Pet. ether refers to the petroleum ether B.p. fraction 60-80°.

Optical rotations were measured in chloroform at room temperature (30 ± 2°) on a Haensch-Schmidt Polartronic I Polarimeter.

U.V. spectra were taken on Perkin-Elmer spectrophotometer model 402, in 95% ethanol. IR spectra were recorded as smears (liquid) or in solution (CCl_4) or in Nujol (solids) on a Perkin-Elmer grating spectrometer model 267. PMR spectra were taken in 10% solution in CCl_4, on a Perkin-Elmer 90 MHz spectrometer, model R32 with TMS as internal standard; signals are recorded in (ppm) relative to TMS as zero. While citing PMR data the following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet, m, multiplet; b, broad.

Mass spectra were determined on a Varian Mat CH7 Mass spectrometer using an ionizing voltage of 70 eV and a direct inlet system, besides the molecular ion, eight most abundant ions, above m/e 50 are reported with their relative intensities.

GLC analysis were carried out on Hewlett-Packard model 5712A Aluminum columns (72" x 1/4" O.D) packed with 10% SE-30 on chromosorb W (60-80 mesh) unless otherwise stated. Hydrogen was used as the carrier gas, at 60 ml/min flow rate. Preparative GLC was carried out on Hewlett Packard model 7624A using Al. columus (144" x 3/8" O.D) packed with 5% carbowax on chromosorb W (60-80 mesh) at 100 ml/min hydrogen flow.

Silica gel for column chromatography (100-200 mesh) and for flash chromatography (200-350 mesh) activated at 125-130^{o} for 6-8 hrs and standardised.[15] TLC was carried out on silica gel (-200 mesh) layers (0.3 mm) containing 10% gypsum, visualization of the spots was carried out with 1% Vanillin in H_3Po_4 aq. (30%) or conc. H_2SO_4 spray followed by heating at around 120^{o}C for 10 mins. Preparative TLC was carried out on 1 mm thick silica gel places.

Medium pressure liquid chromatography (MPLC) was carried out on a Altex glass column (100 x 2.5 cm I.D) packed with active Woelm 32-63 grade silica gel at 40 psi pressure at a flow rate of 10-15 ml/min.

High pressure liquid chromatography (HPLC) was carried out on DuPont 848 instrument with 4.6 mm x 25 cm stainless steel columns packed with zorbax-sil packing. Preparative HPLC was carried out on the same instrument with 2.5 x 44 cm column packed with Spherosil packing.

All solvent extracts were washed with brine and dried over anhydrous sodium sulfate before the solvent removal. Ether refers to diethyl ether.

Extraction of wood

Deodar wood dust* (12 kg) was cold extracted with acetone at 30-32°C and acetone removed using a cyclon evaporator at 38°C under vacuum (200 mm) to give a thick brownish liquid (2.2 kg; 18.3%).

Broad separation

The acetone extract (900 gm) of C. deodara wood dust was thoroughly mixed with celite (1.8 kg) and cold extracted with

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*The wood of C. deodara was obtained from a 10 year old felled stump in Handwara region of Kashmir. The wood was cut into small chips and later pulverised in a ball mill to give the dust.

Petroleum ether to furnish after solvent removal a dark brown, pleasant smelling extract (590 g; 65.5%).

The petroleum ether extract (550 g) as obtained above was taken in light petroleum ether (1L) and cold extracted (5°C) with 5% NaOH (5 x 1 l). The organic phase was washed with water (2 x 1 l) and the washings combined to give NaOH extract (A). The combined aq. phase (A) was washed with ether (2 x 1 l) to remove any non-acidic material and the washings combined with the organic phase. The combined organics were dried (anhyd. Na_2SO_4) and solvent stripping at 38°C gave brownish coloured liquid (504.9 g) kept aside as neutrals.

The aq. extract (A) was cooled to 0° and acidified with aq. phosphoric acid at 0° ± 2°C till the extract was acidic to litmus. It was next cold extracted (0° ± 4°C) with ether (10 x 1 l) and the ether layer dried (anhyd. Na_2SO_4). Removal of solvent furnished the acids as dark brown gum (30 gms).

Esterification of total acids.

The acidic part (30 gms) was dissolved in ether (300ml) and the solution cooled to 0°. Ethereal solution of

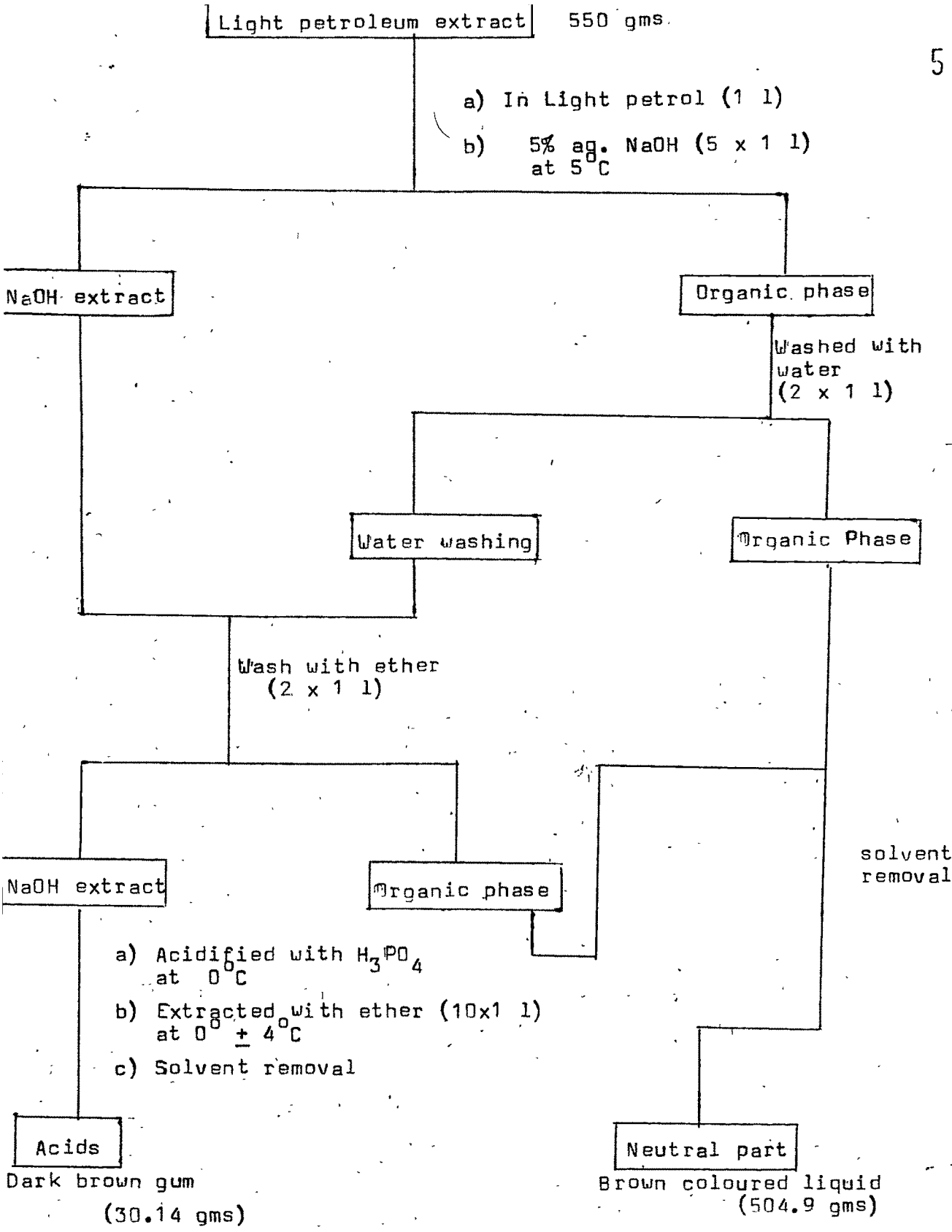

Chart 2. Broad separation of Light Petroleum extract of C. deodara

diazomethane (CH_2N_2) was added in small portions (10% excess) with shaking at 0-5°C until the gas evolution ceased. The reaction mixture was next kept for 2 hours at 0-5°C. Removal of ether furnished the methyl ester (29.4 gms) as a dark brown gum.

Chromatography of the total ester part

The total ester (12.1 gms) were chromatographed on silica gel III A (250 gms; 3.5 x 60 cm) and the following fractions were obtained:

Chromatogram - I

Fr. No.	Eluent	Vol. of eluent (ml)	Weight (g)
1	Pet. ether	5 x 250	-
2	5% $CHCl_3$/pet. ether	8 x 250	1.9078
3	10% $CHCl_3$/pet. ether	6 x 250	0.0530
4	20% $CHCl_3$/pet. ether	6 x 250	
5	1:1 $CHCl_3$/pet. ether	3 x 250	0.0474
6	1:1 $CHCl_3$/pet. ether	11 x 250	3.7238
7	$CHCl_3$	2 x 250	1.2428
8	$CHCl_3$	5 x 250	2.0812
9	10% EtOAc/$CHCl_3$	9 x 250	0.5837
10	10% EtOAc/$CHCl_3$	5 x 250	0.7500
11	MeOH	6 x 250	1.7093
	Total recovery		12.0990 g

No other fraction than Fr. 2 gave any pure compound on repeated chromatography. Fr. 2 (0.6900 gms) was

rechromatographed on silica gel IIB (55 gms; 136 x 1 cm) and following fractions collected.

Chromatogram -II

Fr.No.	Eluent	Vol. of eluent (ml)	Weight (g)
1	Pet. ether	5 x 100	-
2	1% $CHCl_3$/pet. ether	5 x 100	-
3	2% $CHCl_3$/pet. ether	5 x 100	-
4	3% $CHCl_3$/pet. ether	5 x 100	-
5	4% $CHCl_3$/pet. ether	5 x 100	-
6	5% $CHCl_3$/pet. ether	5 x 100	-
7	7% $CHCl_3$/pet. ether	5 x 25	0.1001
8	7% $CHCl_3$/pet. ether	13 x 25	0.0848
9	9% $CHCl_3$/pet. ether	13 x 25	0.0257
10	12% $CHCl_3$/pet. ether	11 x 25	0.0258
11	15% $CHCl_3$/pet. ether	11 x 25	0.0693
12	20% $CHCl_3$/pet. ether	25 x 25	0.1307
13	50% $CHCl_3$/pet. ether	10 x 25	0.0222
14	$CHCl_3$	10 x 25	0.0332
15	EtOAc	100 x 5	0.0963
		Total	0.5881 g

Fr. 8 on crystallization with acetonitrile gave a white crystalline solid (0.0426 g; m.p. 56-57°C) identical on the basis of physical and spectral (NMR, IR, Mass) characteristics with methyl tetracosanoate (VIII).

Fr. 9-12 of chromatogram - II were homogenous and on the basis of physical (tlc) and spectral (NMR, IR, Mass) characteristics were identified as methyl oleate (VII). All other fractions were impure.

Distillation of esters.

Total esters (12 gms) were distilled at 100-240°(bath)/ 0.005 mm to give the distilled esters (8 gms) and residue (3.8 gms).

Medium pressure liquid chromatography of the esters.

Distilled esters (7.8 gms) were chromatographed by loading on a pre-column (2 x 25 cm) and eluting at 40 psi through a larger column (100 x 2.5 cm).

Chromatogram - III

Fr.	Eluent	Vol. of eluent (ml)	Weight (g)
1	Chloroform	7 x 40	0.0082
2	Chloroform	3 x 40	0.2668
3	Chloroform	1 x 40	0.6176
4	Chloroform	1 x 40	3.6041
5	Chloroform	2 x 40	2.6517
6	10% ethyl acetate/ chloroform	5 x 40	0.2500
7	EtOAc	12 x 40	0.2100
		Total	7.6084 g

Fr. 4 (2.4 gms) of the above chromatogram was rechromatographed on silica gel IIA (60 gms; 2.5 x 22 cm) and following fractions collected:

Chromatogram - IV

Fr. No.	Eluent	Vol. of eluent (ml)	Weight (g)
1	Pet. ether	10 x 50	-
2	2% ether/pet. ether	7 x 50	0.0918
3	4% ether/pet. ether	6 x 50	0.0982
4	4% ether/pet.ether	10 x 50	0.5486
5	4% ether/pet. ether	6 x 50	0.5778
6	4% ether/pet. ether	4 x 50	0.2036
7	8% ether/pet. ether	8 x 50	0.1000
8	10% ether/pet. ether	8 x 50	0.0615
9	20% ether/pet. ether	4 x 50	0.0704
		Total	1.7519 g

(+)-Methyl 4(R)-limonene-8(E)-9-carboxylate (IX)

Fr. 3 of chromatogram-IV was found homogenous on tlc (R_f 0.65, 50% ether-pet. ether) and was distilled, b.p. 130-135°(bath)/8 mm $n_D^{30.5}$: 1.4810: found identical (IR, NMR) with the methyl ester of limonenecarboxylic acid M^+ m/e

194 (30.5%); 94 (100%); 101 (41.6%); 85 (29.1%); 93 (27.7%); 87 (19.4%); 68 (13.9%); 81 (13.7%). 57, 135 (12.5%); 102 (11.1%).

7(8)-Dehydrojuvabion (X)

Fr. 5 of chromatogram-IV even though homogenous on tlc (50% ether/pet. ether) and GLC (10% SE-30, 200°C) was found to be impure on HPLC (zorbax sil MeOH-hexane 254 nm UV detection).

It was purified by preparative HPLC. This fraction was injected (60-70 ul as 1% solution in MeOH saturated hexane) on the column *via.* a loop (100 ul) and eluted at ambient temperature (30°C) with methanol saturated hexane at a rate of 60 ml/min (pressure 1000 psi; attenuation 16; chart speed 1"/min). The eluent was detected by a UV cell (254 nm) to give after solvent removal HPLC, GLC and TLC pure ester (R_f. 0.55; 50% ether/pet. ether) identified as 7(8)-dehydrojuvabion(X).

B.p. 160-165°(bath)/0.6 mm , n_D^{30} : 1.4980

$[\alpha]_D^{25}$ + 23.4°($CHCl_3$.C 1%) UV_{max}^{EtOH} 237 nm (ε= 11,040)

M^+: m/e 264^+ (23%); 207 (100%); 59 (54%); 95 (39.1%); 147 (35.1%); 85 (32.4%); 57 (31%); 204 (29.7%); 119, 232 (24.3%). (Found; C, 72.52; H, 9.107; $C_{16}H_{24}O_3$. requires C, 72.72; H, 9.09).

Methyl geronate(XI)

Fr. 7 of chromatogram-IV was next purified on preparative GLC to give a GLC, TLC pure ester (R_f 0.45; 50% ether/pet. ether) identified as methyl geronate XI, b.p. 135-140 bath/10 mm.

M^+: m/e 186(10%); 43 (100%); 102 (66.1%); 69(63%); 73(40%); 129 (32.3%); 109 (27%); 87 (20%); 55,83 (18.4%); 71 (17%); 59 (16%); 111 (14.6%); 154 (10.7%).

Methyl 4-acetyl-1-cyclohexene-1-carboxylate(XII)

Fr. 8 of chromatogram-IV even though a single compound by tlc was found to be only 92% pure on GLC (SE-30, 200°C). B.p. 145-150° (bath)/5 mm. $n_D^{29.5}$: 1.4913 $[\alpha]_D^{25}$ + 3.8° ($CHCl_3$, C. 3%).

M^+: m/e 182 (1.02%); 43 (100%); 139 (79.16%); 79 (54.01%); 107 (50%); 150 (41.66%); 59 (16.66%); 108 (1.02%), 80 (1.00%).

Separation of acids with sodium carbonate

Acetone extract (450 gms) of C. deodara wood dust was thoroughly mixed with celite (900 gms) and cold extracted with pet. ether to furnish after solvent removal the pet. ether extract (327 gms).

The above extract (327 gms) was taken in light petroleum ether (1 l) and cold extracted (0°) with 5% Na_2CO_3 (6 x 500 ml). The organic phase was washed with water (2 x 500 ml) and the washings combined to give the Na_2CO_3 extract (B). The combined aq. phase (B) was washed with ether (2 x 500 ml) to remove any non-acidic carried over material and the washings combined with the organic phase. The combined organics were dried (anhyd. Na_2SO_4) and solvent stripping at $38^{\circ}C$ gave brownish coloured liquid (293 gms) kept aside as neutrals.

The Na_2CO_3 extract (B) was cooled to $0^{\circ}C$ and acidified with aq. phosphoric acid at $0^{\circ} \pm 1^{\circ}C$ till the extract was acidic to litmus. It was next cold extracted ($0^{\circ} \pm 1^{\circ}C$) with ether (6 x 500 ml) and the ether layer dried (anhyd. Na_2SO_4). Removal of solvent furnished acids as dark brown

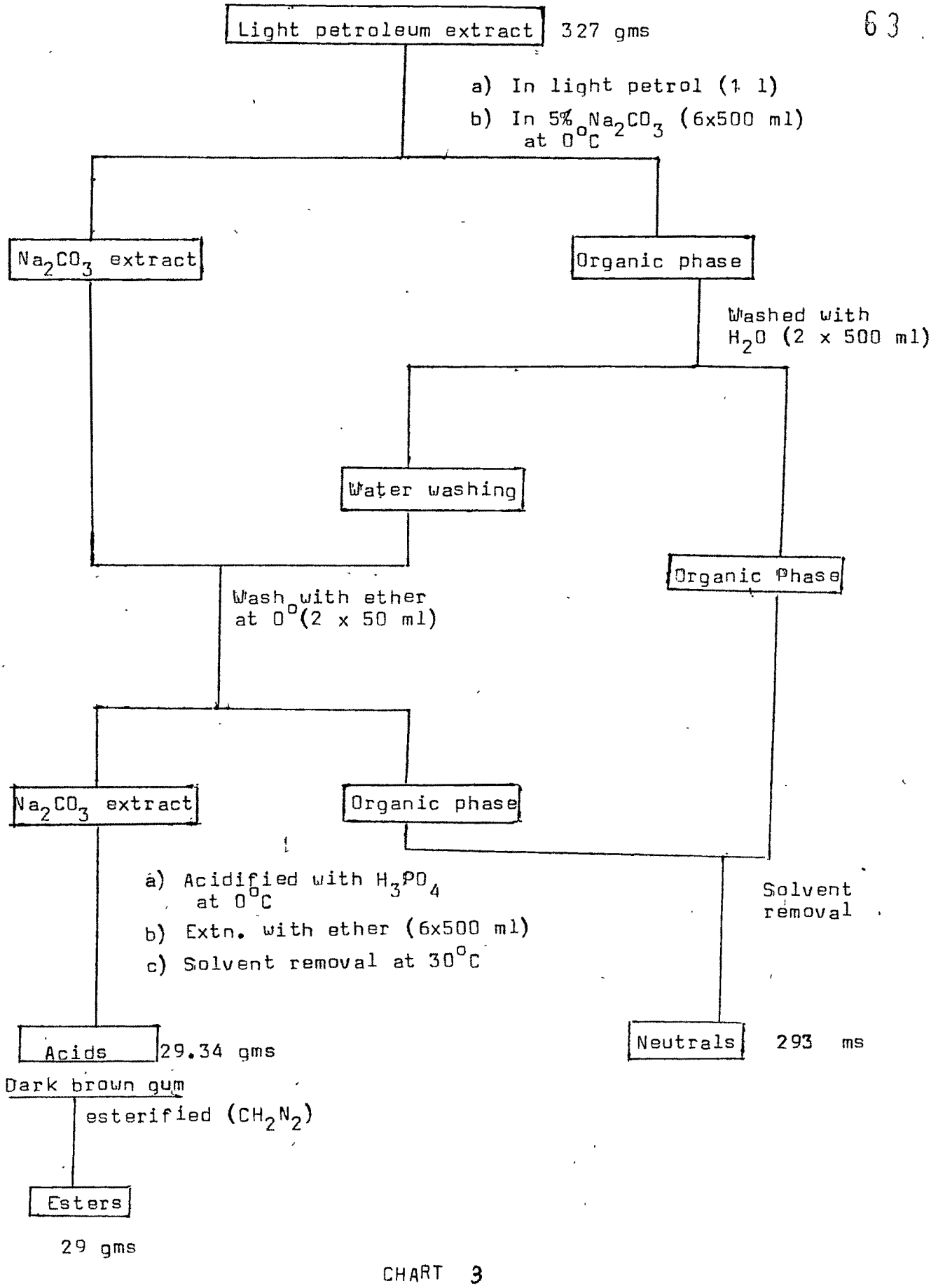
Light petroleum extract
327 gms
a) In light petrol (1 l)
b) In 5% Na_2CO_3 (6x500 ml) at 0°C
Na_2CO_3 extract
Organic phase
Washed with H_2O (2 x 500 ml)
Water washing
Organic Phase
Wash with ether at 0°(2 x 50 ml)
Na_2CO_3 extract
Organic phase
a) Acidified with H_3PO_4 at 0°C
b) Extn. with ether (6x500 ml)
c) Solvent removal at 30°C
Solvent removal
Acids
29.34 gms
Dark brown gum
esterified (CH_2N_2)
Esters
29 gms
Neutrals
293 ms

CHART 3

gum (29.34 gms). The acids thus obtained were immediately esterified (CH_2N_2 method).

Flash chromatography of total esters.

Total esters (1.5 gms) were chromatographed on silica gel (IV-A) (20 gms; 1.5 x 30 cm) and following fractions collected:

Chromatogram -V

Fr.	Eluent	Vol. of eluent (ml)	Weight (g)
1	Pet. ether	3 x 40	0. -
2	Pet. ether	5 x 40	0.2404
3	2% ether in pet. ether	5 x 40	0.0495
4	5% ether in pet. ether	4 x 40	0.2355
5	10% ether in pet.ether	5 x 40	0.0457
6	20% ether in pet.ether	2 x 40	0.0577
7	20% ether in pet.ether	2 x 40	0.0967
8	Ether	2 x 40	0.5151
		Total	1.2406 gms

Fr. 7 was found rich in hydroxyl content (vide IR) and purified next by preparative tlc (solvent system: 50% ether/pet. ether) to afford 0.0323 gms of the pure alcohol (XIII) $n_D^{29.5}$ 1.4942; λ_{max}^{EtOH} : 221 (ε=9815) M^+: m/e 278 (2.3%); 85 (100%); 57 (50%); 140, 143 (54.7%); 164 (16.6%); 79, 105 (14.3%); 180 (13%); 151, 179 (11.9%); 260 (10.7%); 237 (4.3%).

Dehydration of tertiary alcohol XIII

Tertiary alcohol XIII (26 mg) was adsorbed on silica gel IIA (pH; 4; 2 gms) and allowed to stand for 2 hrs. Extraction with ether (10 ml) followed by solvent removal gave a product (18 mg) identified as 7(8)-dehydrojuvabion (X) by TLC, GLC, IR and NMR.

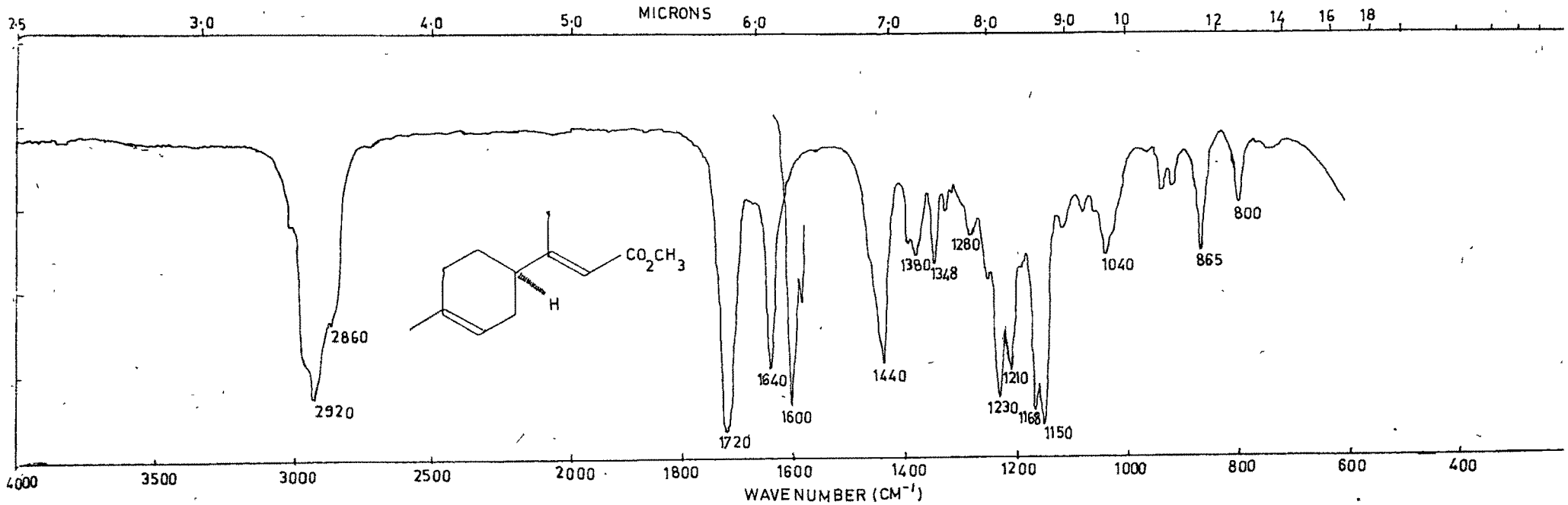

FIG II-1: IR SPECTRUM OF (+)-METHYL 4(R)-LIMONENE-8(E)-CARBOXYLATE (IX)

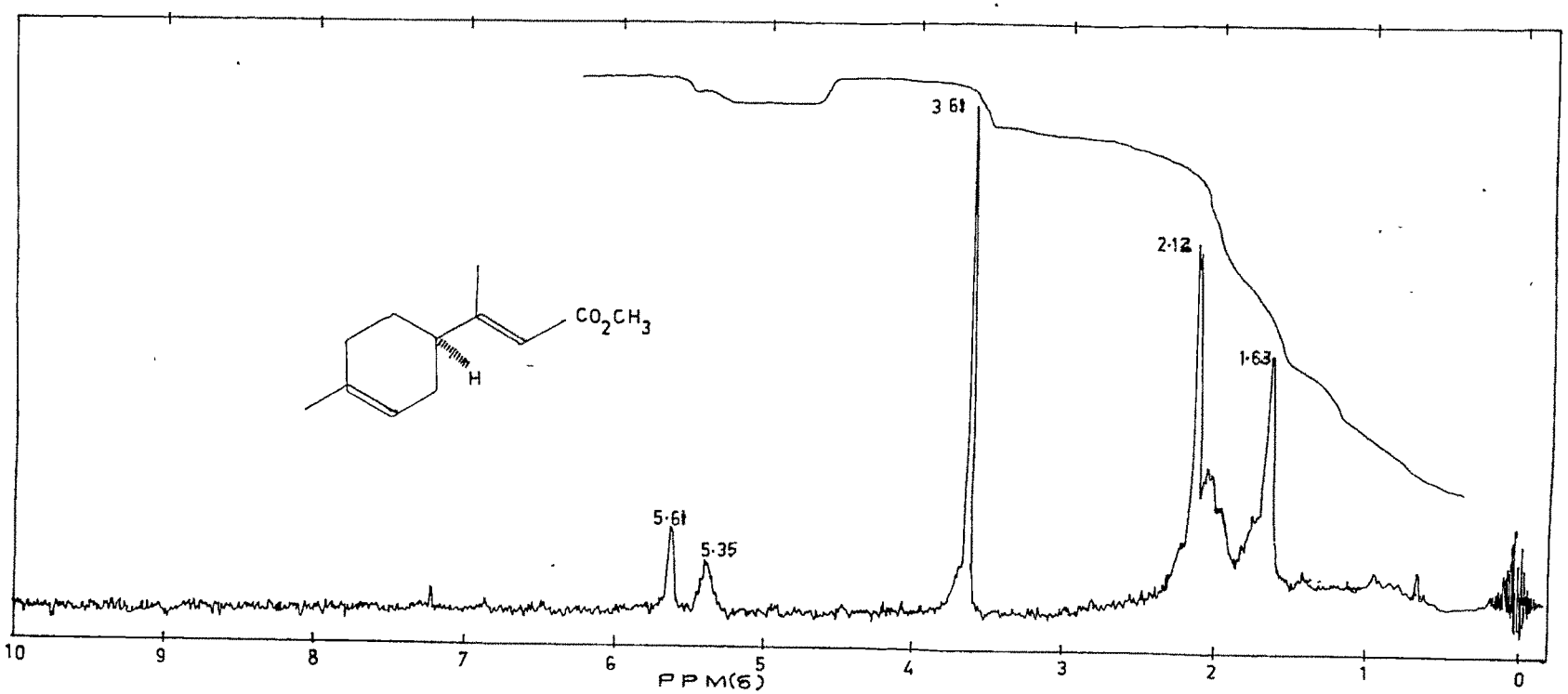

FIG II-2: PMR SPECTRUM OF (+)-METHYL 4(R)-LIMONENE-8(E)-CARBOXYLATE (IX)

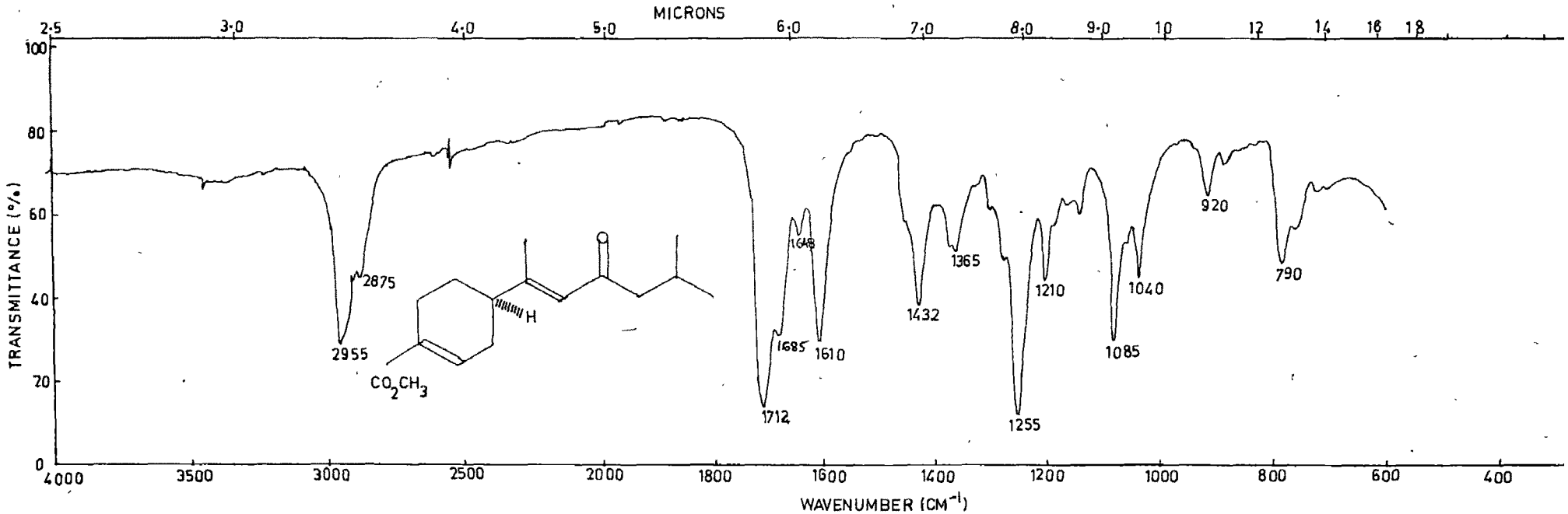

FIG II-3: IR SPECTRUM OF (E)-7 (8) - DEHYDROJUVABION (X)

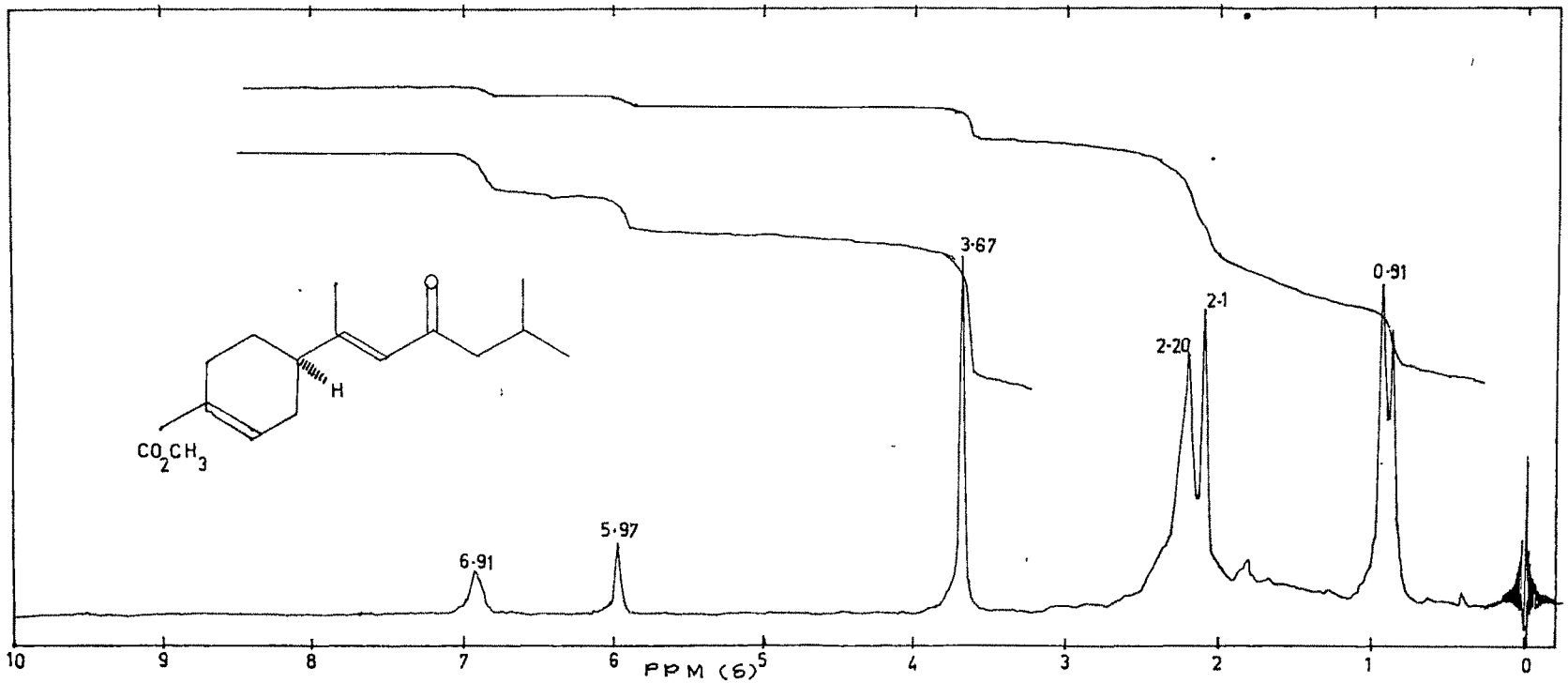

FIG II-4 : PMR SPECTRUM OF (E)-7(8)-DEHYDROJUVABION (X)

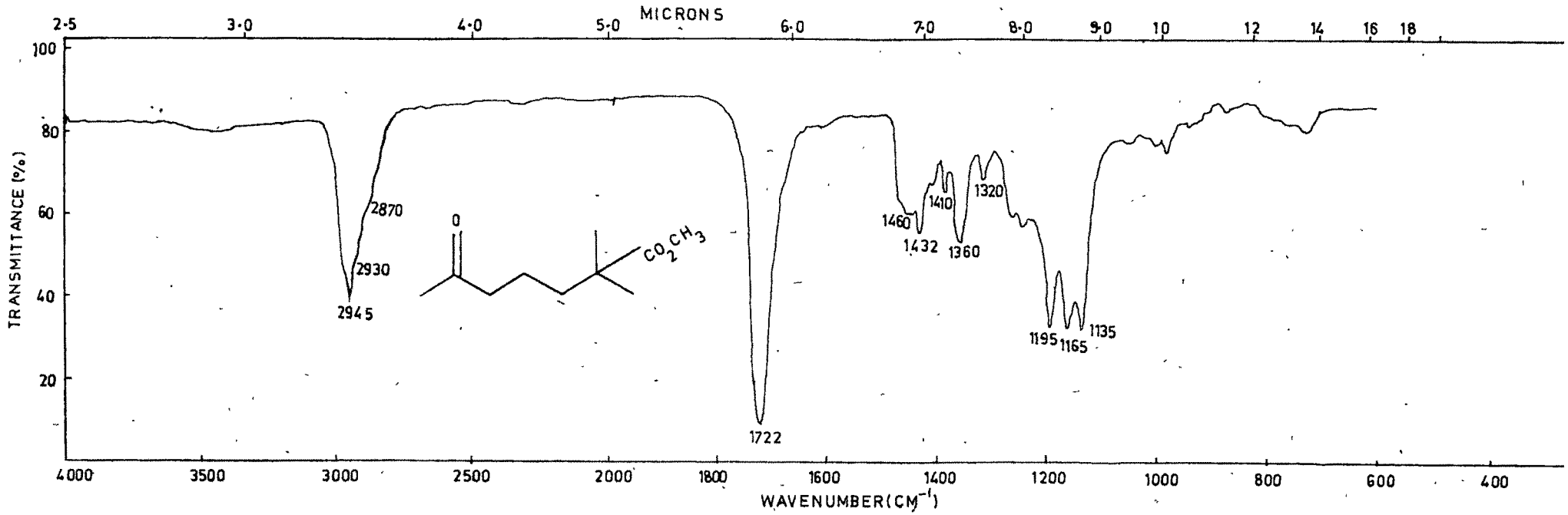

FIG II-5: IR SPECTRUM OF METHYL GERONATE (XI)

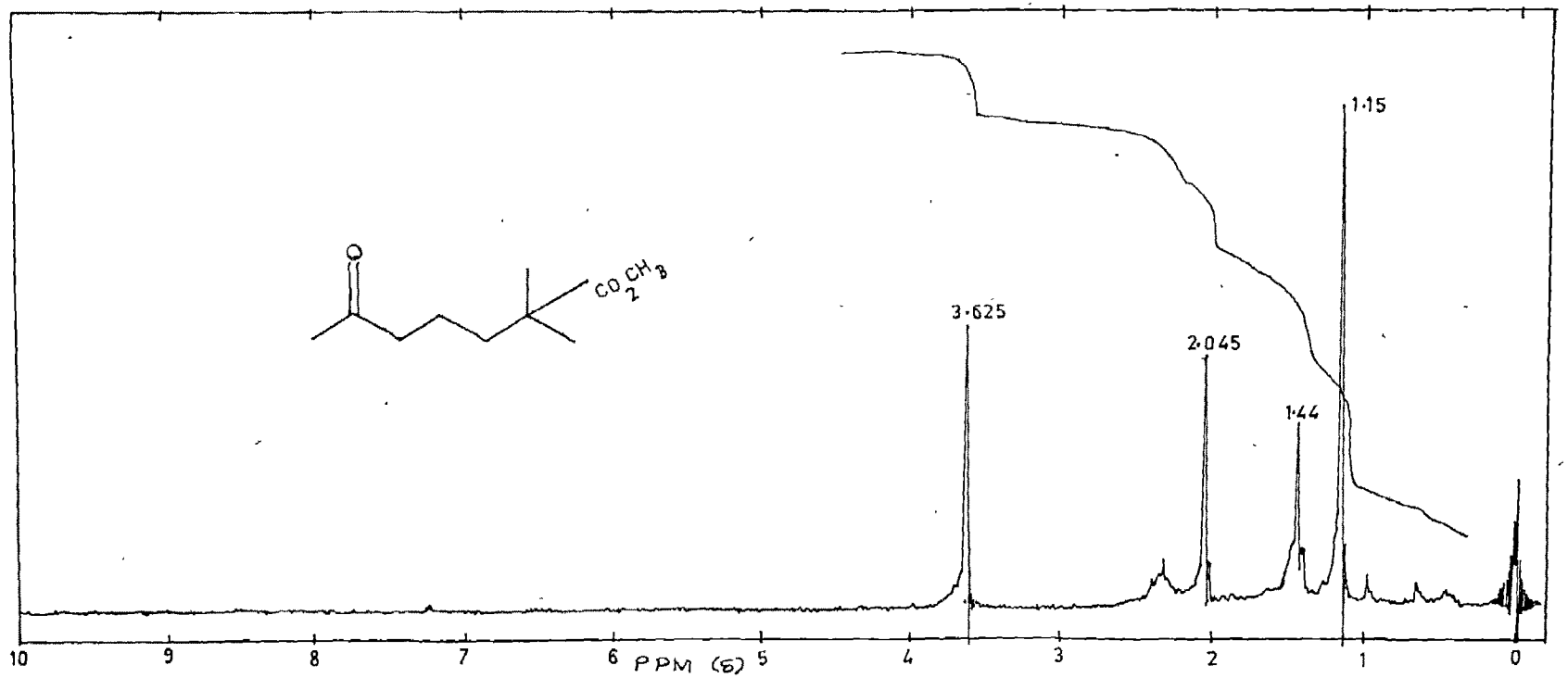

FIG II-6: PMR SPECTRUM OF METHYL GERONATE (XI)

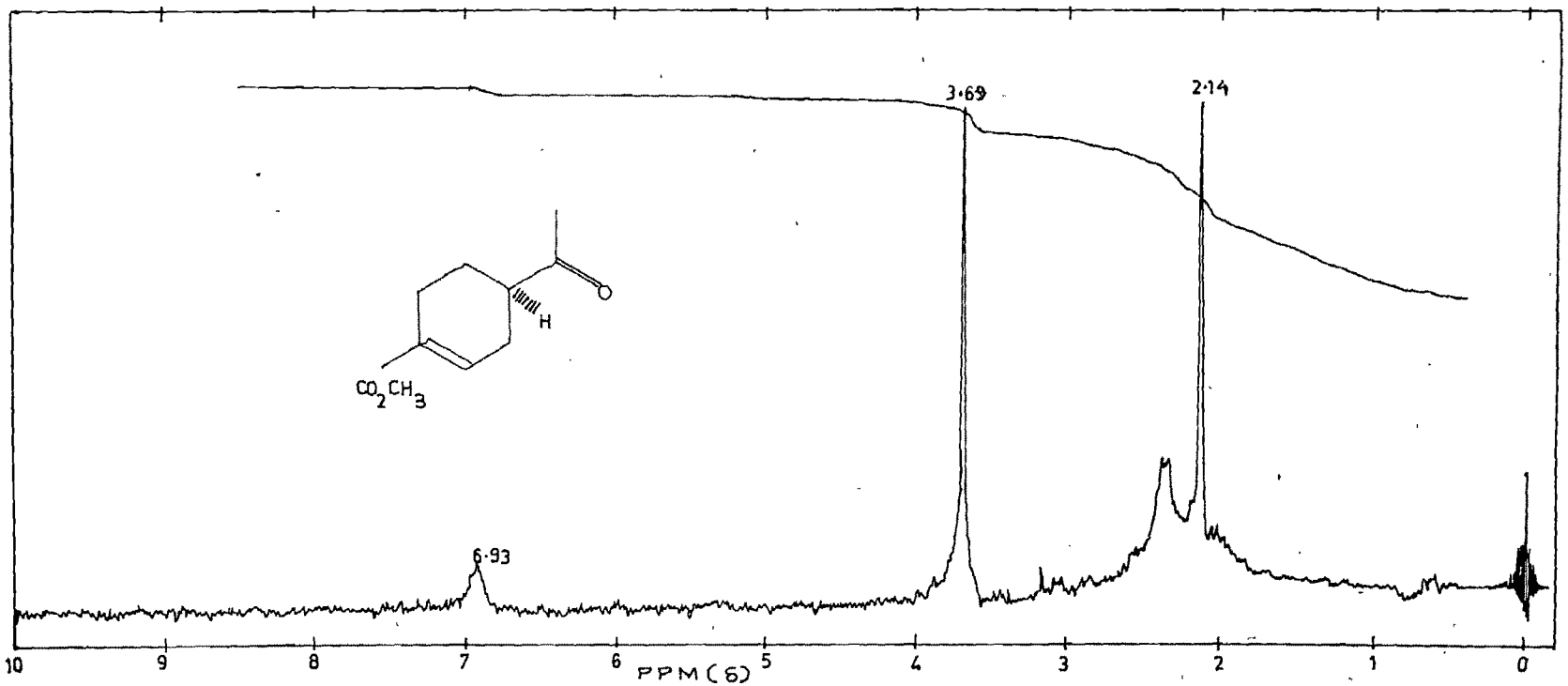

FIG II-7: PMR SPECTRUM OF METHYL 4-ACETYL-1-CYCLOHEXENE CARBOXYLATE (XII).

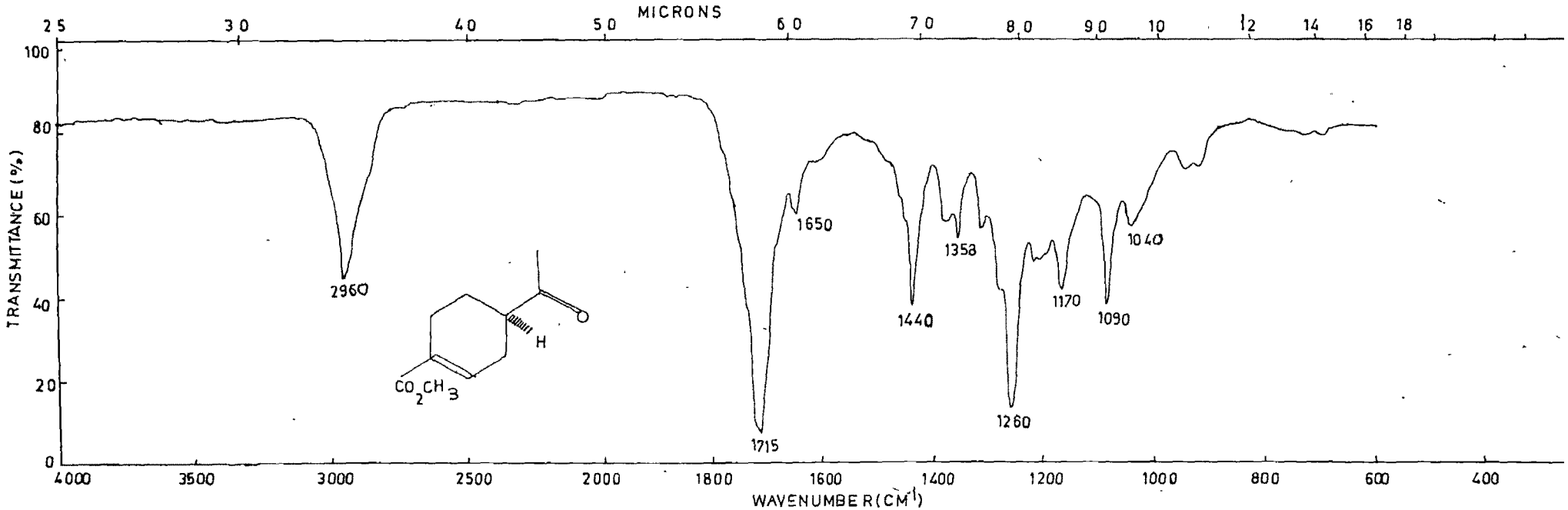

FIG II-8: IR SPECTRUM OF METHYL 4-ACETYL-1-CYCLOHEXENE CARBOXYLATE (X

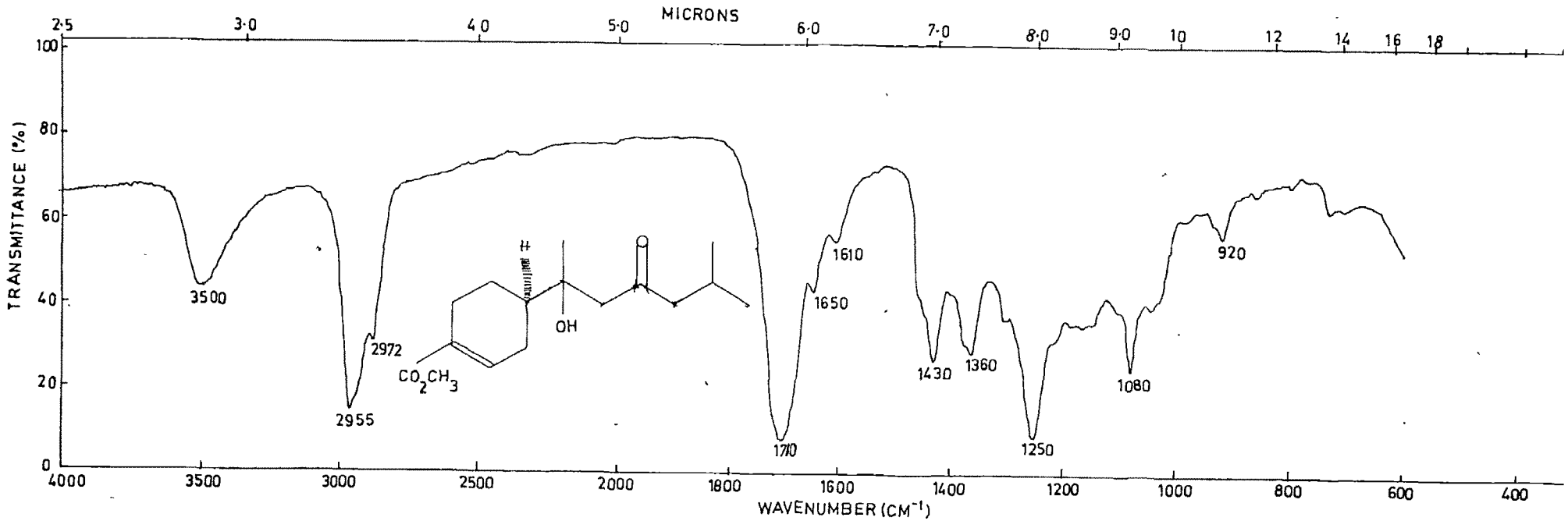

FIG II-9 : I R SPECTRUM OF 7-HYDROXY JUVABION (XVII)

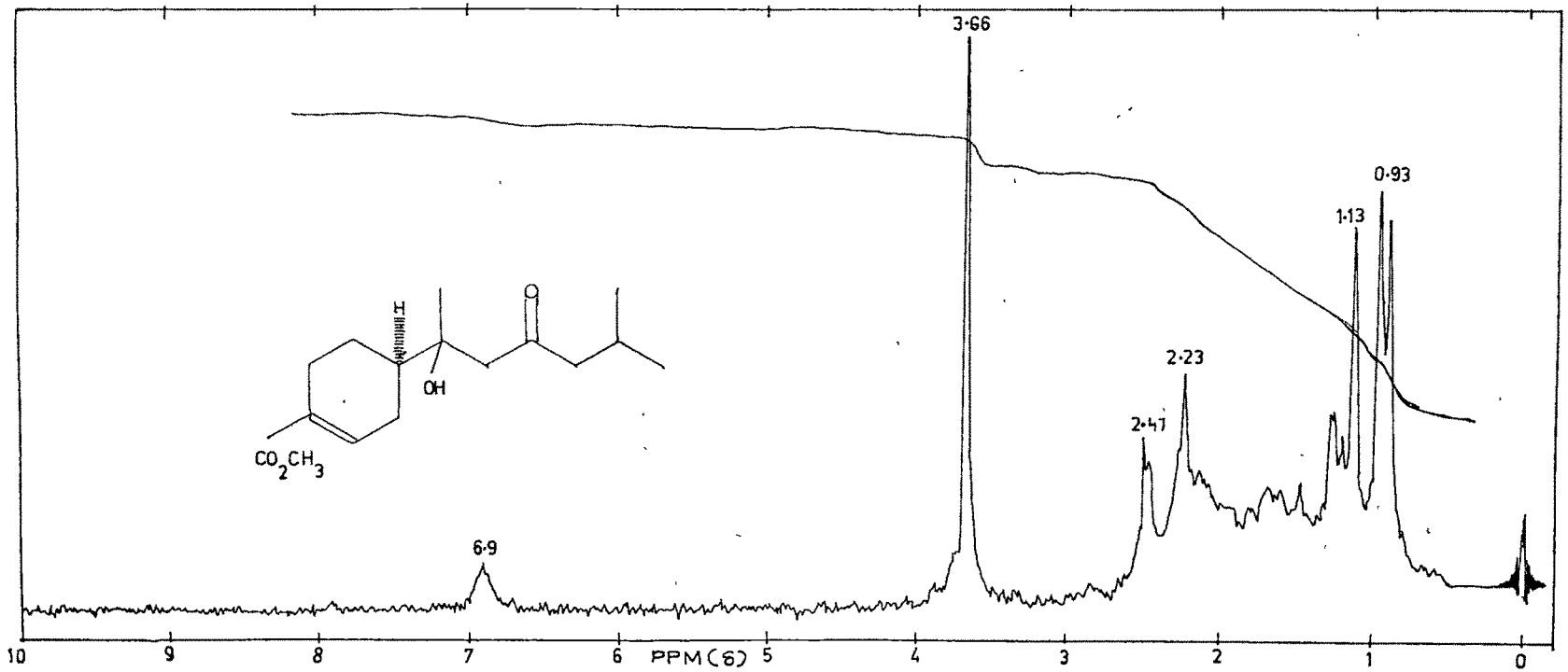

FIG II-10 : P.M.R SPECTRUM OF 7-HYDROXY JUVABION (XVII)

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